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PB-287 601

Fecal Indicator Bacteria Persistence Under Natural Conditions in an Ice-Covered River

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18 May 76

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3.
EPA-600/J-76-082	JOURNAL ARTICLE	PB 287601

4. TITLE AND SUBTITLE
Fecal Indicator Bacteria Persistence Under Natural Conditions in an Ice-Covered River

5. REPORT DATE

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Arctic Environmental Research Station
U. S. Environmental Protection Agency
Fairbanks, Alaska 99701

10. PROGRAM ELEMENT NO.
1BA021

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
same

13. TYPE OF REPORT AND PERIOD COVERED
Interim

14. SPONSORING AGENCY CODE
EPA/ORD

15. SUPPLEMENTARY NOTES
Published in Applied and Environmental Microbiology October 1976, p. 527-536

16. ABSTRACT
Total coliform (TC), fecal coliform (FC), and fecal streptococcus (FS) survival characteristics, under natural conditions at 0°C in an ice-covered river, were examined during February and March 1975. The membrane filter (MF) technique was used throughout the study, and the multiple-tube (MPN) method was used in parallel on three preselected days for comparative recovery of these bacteria. Survival was studied at seven sample stations downstream from all domestic pollution sources in a 317-km reach of the river having 7.1 days mean flow time (range of 6.0 to 9.1 days). The mean indicator bacteria densities decreased continuously at successive stations in this reach and, after adjustment for dilution, the most rapid die-off was found to occur during the first 1.9 days, followed by a slower decrease. After 7.1 days, the relative survival was TC < FC < FS with 8.4%, 15.7% and 32.8% of the initial populations remaining viable, respectively. These rates are higher than previously reported and suggest that the highest survival rates for these bacteria in receiving streams can be expected at 0°C under ice cover. Additionally, the FC-FS ratio was > 5 at all stations, indicating that this ratio may be useable for determining the source of fecal pollution in receiving streams for > 7 days flow time at low water temperatures. Comparison of MPN and MF methods was done in conjunction with that study. The results of this study indicate that heat shock is a major factor in suppression of the FC counts on the membrane filters at 44.5°C. Heat shock may be minimized by extended incubation at 35°C before exposure to the higher temperature.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS		b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Bacteria	Sub-Arctic		06/G,K,M, 08/H,L, 13/B
ollution	Temperature		
cator(s)	Methodology		
Coliform	Water		
Coliform	Rivers		
Streptococci	Ice-Cover		
ic	Survival		

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21. NO. OF PAGES
12

20. SECURITY CLASS *(This page)*
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22. PRICE
A02-A01

PA Form 2220-1 (9-73)

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EPA-600/J-76-082
JOURNAL ARTICLE

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1976, p. 527-536
Vol. 32, No. 4
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Fecal Indicator Bacteria Persistence Under Natural Conditions in an Ice-Covered River

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Received for publication 18 May 1976

Total coliform (TC), fecal coliform (FC), and fecal streptococcus (FS) survival characteristics, under natural conditions at 0°C in an ice-covered river, were examined during February and March 1975. The membrane filter (MF) technique was used throughout the study, and the multiple-tube (MPN) method was used in parallel on three preselected days for comparative recovery of these bacteria. Survival was studied at seven sample stations downstream from all domestic pollution sources in a 317-km reach of the river having 7.1 days mean flow time (range of 6.0 to 9.1 days). The mean indicator bacteria densities decreased continuously at successive stations in this reach and, after adjustment for dilution, the most rapid die-off was found to occur during the first 1.9 days, followed by a slower decrease. After 7.1 days, the relative survival was TC < FC < FS, with 8.4%, 15.7%, and 32.8% of the initial populations remaining viable, respectively. These rates are higher than previously reported and suggest that the highest survival rates for these bacteria in receiving streams can be expected at 0°C under ice cover. Additionally, the FC-FS ratio was >5 at all stations, indicating that this ratio may be useable for determining the source of fecal pollution in receiving streams for >7 days flow time at low water temperatures. The MPN and MF methods gave comparable results for the TC and FS at all seven sample stations, with both the direct and verified MF counts within the 95% confidence limits of the respective MPNs in most samples, but generally lower than the MPN index. Although FC recovery on membrane filters was improved with a modified technique, the MPN and MF methods did not provide comparable results at stations near the pollution source. However, the results became more comparable with increasing flow time. The results of this study indicate that heat shock is a major factor in suppression of the FC counts on the membrane filters at 44.5°C. Heat shock may be minimized by extended incubation at 35°C before exposure to the higher temperature.

Water temperature, chemical and physical parameters, and predation are among the major factors affecting enteric microorganism survival in the aquatic environment (15, 19, 22, 30). Early in this century, Ruediger (35) demonstrated a seasonal effect on survival. Subsequent studies confirmed his observation that greater numbers of fecal indicator bacteria survive in water for a longer time during the winter than during the summer (7, 18, 21, 25, 32). Based on a literature review, Kittrell and Furfari (25) suggested 15°C as an adequate dividing point for determining seasonal water temperature effects. However, the work of McFeters and Stuart (30) suggested this division may not be adequate when they found that the temperature effect on *Escherichia coli* survival time was inversely proportional to the temperature change between 5 and 15°C. Since *E. coli* has been shown to have a minimum growth temperature of 7.5 to 7.8°C (39), the low-temperature survival picture may be clouded by the coliform aftergrowth demonstrated in some studies at temperatures above the minimum for growth but less than 15°C (25, 31). Although fecal streptococci (FS) survive longer in winter than in summer (7, 42), aftergrowth does not appear to be a problem since these bacteria rarely, if ever, multiply in water (4, 13).

Most fecal indicator bacteria survival studies have been conducted under controlled laboratory or field conditions utilizing pure cultures of coliforms and FS. Such controlled studies contribute immeasurably to a basic understanding of survival characteristics (2, 29) and are necessary to delineate the effect of individual factors such as water temperature (14, 30). However,

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extrapolation to natural conditions must be done with great caution because controlled studies cannot take into account all the environmental factors and interrelationships in natural aquatic ecosystems (11, 12, 14, 29).

Enteric microorganism survival in receiving streams is one of the critical factors impacting downstream water uses (14). Since low water temperatures enhance survival and prevail over a large part of the world for a portion of each year, low-temperature survival parameters must be established in natural receiving waters. An earlier report (18) on fecal indicator bacteria survival characteristics at low water temperatures pointed out the need to relate indicator and pathogen survival. As a result, the low-temperature survival characteristics of the indicator bacteria, salmonellae, and enteric viruses were examined during an intensive sampling program on the Tanana River near Fairbanks, Alaska, when the water temperature was 0°C and the river was ice covered. The present report describes the fecal indicator bacteria survival characteristics and compares total coliform (TC), fecal coliform (FC), and FS recovery using the membrane filter (MF) technique and multiple-tube (MPN) method.

MATERIALS AND METHODS

River description. The Tanana River, one of the major rivers draining the interior of Alaska, flows generally northwest to its confluence with the Yukon River. The 360-km reach studied (Fig. 1) has complete ice cover and a water temperature of 0°C from November through April. Unlike many rivers in more temperate climates which have high fluctuating flows (25), the Tanana River (U.S. Geological Survey, personal communication) and other Alaskan rivers (37) have low flows during the winter. The minimum flow is usually reached during early January and remains stable until the beginning of spring breakup in April. The Tanana River receives domestic sewage effluent from several waste treatment systems in a reach starting at its confluence with the Chena River and extending upstream for 35 km. Most of this effluent enters from the Chena River which flows through Fairbanks; there is no additional input downstream from this point.

Sample station selection and field schedule. Ten sample stations were selected (Fig. 1). The T-900 station was located upstream from all known domestic waste sources to provide background information. The T-800 and C-100 stations monitored all known sewage sources on the Tanana and Chena Rivers, respectively. The T-700 station was located downstream from the confluence of the Tanana and Chena Rivers, a distance sufficient to permit complete mixing of the water from the two rivers. The T-700 through T-100 stations established the 317-km reach in which the survival study was conducted and, based on previous velocity estimates (18), divided the river into approximately 1-day flow time reaches.

The study was conducted during February and March 1975. The 17 February (day 1) to 7 March (day 19) time period was selected because of stable river discharge and ice conditions, sufficient daylight for field work, and ambient air temperature generally warmer than −30°C. Hydrological studies were started and preliminary bacteriological samples were obtained from each station during days 1 through 7. Days 8 through 19 were utilized for intensive sampling and completion of the hydrological studies.

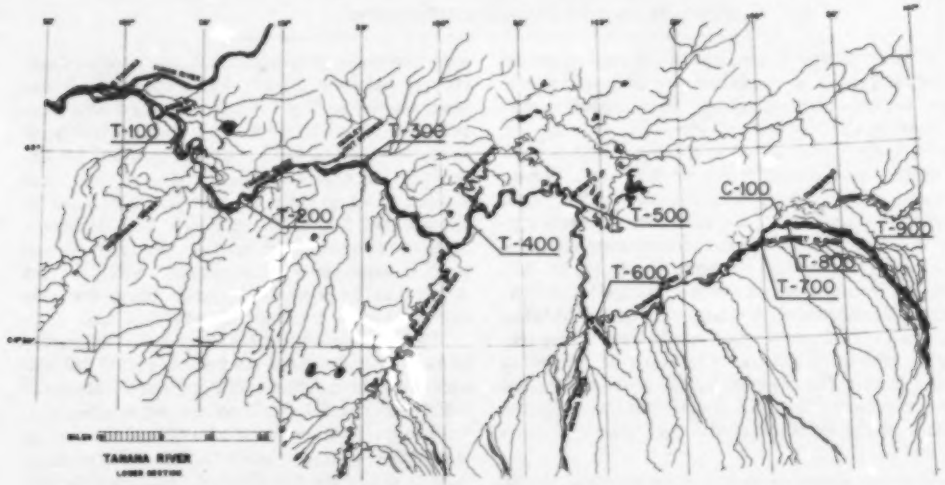

FIG. 1. *Lower reach of the Tanana River showing sample stations.*

Sampling methods. The C-100 sample station was the only one readily accessible by ground transportation. Small, ski-equipped aircraft were used to reach the other stations. The techniques for this type of winter field operations have been described by Casper et al. (6) and Gordon (18).

All samples were collected in new 5 (ca. 19.0 liters)- or 6 (ca. 22.8 liters)-gallon Cubitainers (Hedwin) which were discarded after one use. A new hole was cut in the ice each day that samples were taken. Water for the 6-gallon bacteriology and chemistry samples was dipped from the hole. At stations where larger volumes of water (106 gallons [ca. 402.8 liters]) were required, a submersible pump powered by a small generator was used to pump water through a low-temperature rubber hose directly into Cubitainers inside the aircraft. Each sample was delivered to a small airport near the T-600 station and transferred to an insulated truck for transport to the laboratory. Samples from the T-100 through T-700 stations were collected between 10:00 a.m. and 2:00 p.m. and maintained at a temperature near 0°C during transport. All samples were in the laboratory within 6 h after removal from the river.

Hydrological studies. U.S. Geological Survey hydrologists, experienced in doing under-ice measurements, used established techniques to measure the river discharge at each station. This was used to determine the dilution factor for enteric microorganism survival and for the dye study. The hydrologists also marked the center of the main river channel at each station to establish the dye injection and microbiology sampling points.

The dye study provided the minimum, mean, and maximum flow times between stations. Based on the river discharge, appropriate volumes of Rhodamine B dye were injected at each station from T-200 proceeding upstream through T-700 in consecutive order. The dye passage at each station from T-100 to T-600 was monitored using a Turner model 111 fluorometer fitted with a high-volume continuous flow door. Because it was not advisable to leave a person on the ice during the extended time required for dye passage, a procedure was developed that permitted the monitoring equipment to operate unattended for 24 h or longer. A report on the details of this procedure is in preparation.

Indicator bacteria analysis. Because MF brand and lot may affect TC and FC recovery (10, 20, 33, 36), a preliminary comparison of Millipore filters (type HAWG 047 SO; lot numbers 12463 1, 12463 2, 17668 4 and 17668 5; Millipore Corp.) and Gelman filters (type GN-6; lot number 80689) was conducted. A pure culture of *E. coli* (ATCC 11775) was used for the initial comparisons. Subsequently, unchlorinated primary sewage effluent and polluted river water from the C-100, T-600, and T-700 stations were examined for TC, FC, and FS, and isolates were verified to determine recovery efficiency. The comparison indicated that neither the TC nor the FS counts were affected by MF brand or lot. Incorporation of agar into the medium showed no improvement in TC counts. FC recovery on Gelman filters was higher than the best recovery on Millipore filters (lot number 17668 5, Millipore Corp.) by 1.4 times with *E. coli* and 1.9 times with polluted river water.

Using Gelman filters, additional testing with river water samples showed that the broth medium gave 1.8 to 2.0 times greater FC recovery than the agar medium. It was suggested (R. H. Bordner, personal communication) that FC recovery may be increased by a preincubation step to minimize or prevent heat shock arising from the rapid temperature increase from 0 to 44.5°C. To examine this possibility, water samples were filtered in the normal manner, and the filters were placed on the broth medium and left at either room temperature or 35°C for 2 h. This was followed by incubation in a 44.5°C water bath for 24 h. Parallel samples were immediately placed in the water bath. The counts from preincubated filters were 2.0 to 2.3 (room temperature) and 2.8 to 3.5 (35°C) times greater than those from filters placed immediately at 44.5°C.

The MF technique as described in Standard Methods (1) was used throughout the study for the determination of total coliforms (m-TC), fecal coliforms (m-FC) and fecal streptococci (m-FS), except that the FC filters were preincubated for 2 h at 35°C. Millipore filter lot number 17668 5 was used for both TC and FS, and Gelman filter lot number 80689 was used for FC. The growth media used were m-Coliform broth (BBL), KF streptococcal (KF) agar (BBL) and m-FC broth (BBL). Three replicate portions of each sample volume were filtered. To minimize variables in counting during the study, one person did all MF counting and isolation of colonies for verification.

On days 5, 12, and 19, 10 MF colonies exhibiting positive color reactions were randomly picked from each of the three replicate filters for verification. The TC and FC verification methods utilized during this study have been described elsewhere (41). In addition, TC isolates that showed a positive presumptive test were subcultured in EC broth (Difco) for verification as FC. Verified TC and FC isolates from day 5 were subcultured on Endo agar (BBL) plates and typical coliform colonies were transferred to Trypticase soy agar (BBL) slants for further study. FS verification was an abbreviated procedure using the same media and methods as the MPN. Isolates verified by this procedure on day 5 were subcultured on brain heart infusion agar (BBL) plates, and colonies were transferred to slants on the same medium for further study.

The MPN method (1) was used in parallel with the MF technique on days 5, 12, and 19. Either lauryl sulfate broth (BBL) or lauryl tryptose broth (Difco) was used for the presumptive coliform MPN test. All positive presumptive cultures were transferred to brilliant green bile 2% (Difco) for TC confirmation and to EC medium (Difco) for FC confirmation. Azide dextrose broth (BBL) and ethyl violet azide broth (BBL) were used in the presumptive and confirmed MPN tests for FS.

Chemical analyses. Samples were obtained during the intensive sampling period (days 8–19). Immediately after the samples arrived in the laboratory, pH was determined with a Leeds & Northrup model 7401 pH meter, and conductivity was mea-

sured with a Beckman model RC 16B2 conductivity bridge. A Technicon AutoAnalyzer I and automated methods (40) were used to measure concentrations of the following chemical parameters: chloride, ammonia nitrogen, nitrate nitrogen (hydrazine reduction method), and orthophosphate phosphorus (single reagent method). Calcium, magnesium, sodium, and potassium concentrations were measured with a Perkin-Elmer model 303 atomic absorption spectrophotometer. A Beckman model 915 total organic carbon analyzer was used for measuring total carbon and total organic carbon.

RESULTS AND DISCUSSION

TC, FC, and FS recovery with the MF technique. The data in Table 1 reveal that the Chena River (C-100) was the major source of fecal pollution in the Tanana River. These bacteria are almost entirely of domestic origin during the winter when ice effectively seals the river because there are no other point or nonpoint sources. Thus, the wide range in numbers of indicator bacteria recovered at each station can be attributed mainly to diurnal fluctuations in domestic sewage flow and are comparable to the ranges previously reported for polluted rivers (17, 18, 27). The bacterial counts overlapped extensively between consecutive stations, but there was a continuous decrease in the mean TC, FC, and FS densities as the water moved downstream.

Even though the TC, FC, and FS densities at each station varied widely from day to day, the mean coefficients of variation (Table 2) indicate that the MF technique had a high degree of precision for enumerating indicator bacteria in this type of river water sample. The MF technique was used on a total of 61 samples from stations T-700 through T-100, with overall coefficients of variation of 0.13 (TC), 0.15 (FC), and 0.10 (FS). These values for TC and FC are in agreement with the mean coefficients of variation reported earlier for pure coliform cultures (A. P. Dufour and V. J. Cabelli, presented at the EPA-ASTM Symposium on Membrane Filters, Fort Lauderdale, Fla., 20 January 1975; 33). Thus, the type of laboratory errors described by E. E. Geldreich (presented at the EPA-ASTM Symposium on Membrane Filters, Fort Lauderdale, Fla., 20 January 1975) and Velz (44) can be minimized and excellent reproducibility can be obtained with environmental samples.

Geldreich (13) pointed out that the FC-FS ratio indicates whether pollution is from a human or nonhuman source because it is always >4 in domestic sewage effluent and generally <0.7 in animal wastes. McFeters et al. (29) reported that the persistent FS species are of human origin, so the FC-FS ratio would fall when domestic effluent entered the stream environment and started moving downstream, whereas the ratio in animal wastes would rise. Because of the different rates of FC and FS die-off in natural streams, it has been suggested that the ratio may not be valid as a source indicator after 24 h (13, 27). However, McFeters et al. (29) found the ratio to be valid for as long as 4 days at 9.5 to 12.5°C. The data in Table 3 suggest that the FC-FS ratio may be valid for an even more extended period at 0°C as it was still >5 after 7 days of flow time.

Verification of MF isolates. Verification of TC, FC, and FS isolates is shown in Table 4. Although the mean TC verification was 80.6% at the seven downstream stations, the percentages varied widely among the samples even though the pollution source was known to be domestic sewage. The verification range of 50.0

TABLE 1. *Arithmetic mean and range of fecal indicator bacteria MF counts per 100 ml of sample from each station*

Sample station	TC		FC		FS	
	Mean	Range	Mean	Range	Mean	Range
T-900[a]	5	1–8	<1	<1–2	2	1–6
T-800[b]	49	17–83	10	2–19	6	3–10
C-100[b]	130,000	93,000–220,000	29,000	13,000–47,000	3,400	1,700–5,100
T-700[c]	5,900	2,700–9,100	1,200	430–2,200	99	26–160
T-600[c]	2,200	1,600–3,500	500	290–850	64	39–80
T-500[b]	1,400	700–2,400	360	100–520	45	11–83
T-400[c]	800	500–1,200	250	98–560	36	27–46
T-300[b]	490	230–650	150	100–210	27	16–55
T-200[b]	360	180–560	110	58–160	22	13–47
T-100[c]	290	170–530	110	65–180	19	14–23

[a] Six samples collected (days 1, 8, 10, 12, 15, 19).
[b] Seven samples collected (days 1, 5, 8, 10, 12, 15, 19).
[c] Ten samples collected (days 1, 5, 8, 9, 10, 11, 12, 15, 16, 19).

TABLE 2. *Mean coefficient of variation of MF counts from each sample station*[a]

Sample station	TC	FC	FS
T-900	0.38		0.43
T-800	0.19	0.23	0.22
C-100	0.14	0.21	0.11
T-700	0.13	0.18	0.11
T-600	0.11	0.15	0.09
T-500	0.12	0.17	0.09
T-400	0.13	0.17	0.08
T-300	0.12	0.12	0.10
T-200	0.18	0.12	0.14
T-100	0.13	0.15	0.11

[a] Calculated from the sum of the coefficients of variation obtained each day.

TABLE 3. *Mean TC-FC and FC-FS ratios from MF counts per 100 ml at each sample station*[a]

Sample station	TC-FC	FC-FS
T-900	3.9	0.7
T-800	5.8	1.9
C-100	5.3	8.9
T-700	5.4	13.9
T-600	4.9	8.2
T-500	4.2	8.5
T-400	4.1	7.1
T-300	3.3	6.0
T-200	3.3	5.4
T-100	2.8	5.5

[a] Calculated from the sum of the ratios obtained each day.

to 96.7% for samples from these stations was comparable to that reported for other rivers (9) and supports the conclusion that many noncoliform bacteria are capable of producing a false-positive m-TC reaction (9, 38). Concurrently, these TC isolates were checked for verification as FC and the mean verification rate was 43.5%, with a range of 16.7 to 66.7%. The mean FC verification rate was 92.5% (range, 83.3 to 100%) for m-FC isolates after the 35°C preincubation and incubation at 44.5°C. This is in close agreement with the 91.0% FC verification from river water samples reported by Rose et al. (34). The FS had a mean verification of 94.5% (range, 83.3 to 100%), which was slightly higher than reported previously (8), and may have resulted from the abbreviated verification method used in this study. These data show that verification of m-TC isolates either as TC or FC was highly variable from sample to sample and had no consistent relationship to m-FC isolate verification. In contrast, neither day nor movement downstream from the pollution source affected verification of m-FC or m-FS isolates. The percentages of FC and FS isolates verified were similar in individual samples and had essentially the same overall mean and range.

Comparison of TC, FC, and FS recovery with MF and MPN methods. In some reports, it is difficult to ascertain whether the MPN index or the 95% confidence limits were used to compare the MPN and MF methods. Since the index is an estimate of bacterial density rather than a precise measurement (41), it is of little significance without the confidence limits because any bacterial density within the limits can produce the observed result (44). Thus, both the MPN index and 95% confidence limits were used during this study to compare MF and MPN recoveries of TC, FC, and FS from the Tanana River. Parallel MF and confirmed MPN results are shown in Tables 5, 6, and 7, along with the MF counts adjusted for the percent verification from Table 4. The TC densities (Table 5) show that the m-TC counts were generally lower than the confirmed MPN index. This appears to be in agreement with previous reports, which indicate that the m-TC counts are often lower than the MPN densities in river water samples (25, 28). However, the two methods did provide comparable results, unaffected

TABLE 4. *Verification of MF colonies from each sample station*[a]

Sample station	Day sample taken	TC		FC	FS
		% Verified as TC	% Verified as FC	% Verified	% Verified
T-700	5	90.0	56.7	100.0	96.7
	12	90.0	50.0	96.7	96.7
	19	76.7	30.0	83.3	100.0
T-600	5	86.7	56.7	93.3	93.3
	12	76.7	36.7	90.0	83.3
	19	70.0	36.7	86.7	90.0
T-500	5	90.0	63.3	90.0	96.7
	12	73.3	40.0	100.0	100.0
	19	80.0	33.3	93.3	93.3
T-400	5	90.0	60.0	86.7	86.7
	12	50.0	26.7	93.3	93.3
	19	63.3	40.0	86.7	90.0
T-300	5	90.0	46.7	93.3	86.7
	12	66.7	43.3	93.3	96.7
	19	76.7	16.7	96.7	100.0
T-200	5	96.7	56.7	83.3	83.3
	12	93.3	66.7	96.7	96.7
	19	66.7	20.0	100.0	100.0
T-100	5	90.0	40.0	93.3	96.7
	12	93.3	66.7	96.7	96.7
	19	83.3	26.7	90.0	100.0

[a] Thirty colonies subcultured from each station each day.

TABLE 5. *Comparison of MPN and MF counts of TC per 100 ml from each sample station*

Sample station	Day sample taken	Confirmed MPN		MF		
		Index	95% Confidence limits	Density[a]	Verified as TC	Verified as FC
T-700	5	7,900	2,500–19,000	6,200	5,600	3,500
	12	24,000	6,800–75,000	9,100	8,200	4,600
	19	13,000	3,500–30,000	7,900	6,100	2,400
T-600	5	5,400	1,800–14,000	1,800	1,600	1,000
	12	4,900	1,700–13,000	1,700	1,300	620
	19	3,300	1,100–9,300	1,700	1,200	660
T-500	5	1,700	430–4,900	700	630	440
	12	3,300	1,100–9,300	1,300	950	520
	19	700	230–1,700	710	570	240
T-400	5	700	230–1,700	500	450	300
	12	1,700	430–4,900	1,200	600	320
	19	1,100	310–2,500	690	440	280
T-300	5	170	43–490	230	210	110
	12	490	170–1,300	560	370	240
	19	790	250–1,900	630	480	110
T-200	5	490	170–1,300	180	170	100
	12	1,800	440–5,000	560	520	370
	19	330	110–930	500	330	100
T-100	5	700	230–1,700	200	180	80
	12	790	250–1,900	530	490	350
	19	330	110–930	260	220	69

[a] Mean of three replicate portions.

by downstream movement or by the day the samples were taken.

The relationship of the MF and MPN methods for FC enumeration (Table 6) was not as clear-cut as for TC. Nonlethal injury, preventing growth of a significant fraction of the coliform population on selective media, has been shown to occur (2, 3, 19, 26). These reports indicate that injury is repairable if the cells are first grown in nonselective media at 35°C. The heat-shock stress on both injured and uninjured coliform cells, when subjected to a rapid temperature increase to 35°C, has not been examined in freshwater. This could be particularly severe when the sample temperatures are near 0°C. However, the effect of raising the temperature from 35 to 44.5°C has been studied, and recent reports (10, 20) showed a higher *E. coli* recovery on the m-TC medium at 35°C than on the m-FC medium at 44.5°C. In contrast, Presswood and Brown (33) found that the 44.5°C incubation temperature was not detrimental to several *E. coli* strains; extended 35°C incubation had preceded the temperature increase. Subsequently, a two-layer agar medium was used by Rose et al. (34) to examine the effect of a 2-h preincubation at 35°C on recovery of FC at 44.5°C. This increased recovery of FC in natural environmental samples from a variety of sources. The increased recovery in samples from some sources was attributed to repair of injured cells, but no reason was given for the better recovery from river water samples. Our preliminary investigation showed a similar increase which could only be attributed to reduced impact of heat shock on uninjured cells since preincubation was on the selective m-FC medium.

In this study, it appears that a heat-shock effect on uninjured cells caused by raising the temperature to 44.5°C was a major factor in FC recovery, and may account for the discrepancies between the m-FC and MPN methods (Table 6). Even with the preincubation step, both the verified and unverified m-FC counts were less than the lower 95% confidence limit of the MPN in all but two samples at the T-700 through T-

TABLE 6. *Comparison of MPN and MF counts of FC per 100 ml from each sample station*

Sample station	Day sample taken	Confirmed MPN		MF		
		Index	95% Confidence limits	Density[a]	Verified	TC verified as FC[b]
T-700	5	7,900	2,500–19,000	2,200	2,200	3,500
	12	4,900	1,700–13,000	1,400	1,400	4,600
	19	3,300	1,100–9,300	880	730	2,400
T-600	5	5,400	1,800–14,000	430	400	1,000
	12	2,200	700–6,700	290	260	620
	19	2,300	700–7,000	230	200	660
T-500	5	460	160–1,200	100	90	440
	12	1,300	350–3,000	260	260	520
	19	490	170–1,300	190	180	240
T-400	5	490	170–1,300	200	170	300
	12	1,700	430–4,900	250	230	320
	19	460	160–1,200	100	87	280
T-300	5	170	43–490	100	93	110
	12	220	70–670	150	140	240
	19	170	43–490	150	150	110
T-200	5	130	35–300	68	57	100
	12	1,200	300–2,800	120	120	370
	19	240	68–750	120	120	100
T-100	5	220	57–700	90	84	80
	12	790	250–1,900	190	180	350
	19	130	35–300	96	86	69

[a] Mean of three replicate portions.
[b] From Table 5.

400 stations. The cell densities became more comparable after 5.3 days flow time downstream from the pollution source and were in agreement in all but two samples at stations T-300 through T-100. The verified FC densities from the m-TC medium were within the 95% confidence limits of the MPN in all but 4 of the 21 samples, but lower than the index in all but one sample. Since the m-FC and m-TC media are both selective, the difference in FC recovery may be attributable to the length of incubation at 35°C before exposure to 44.5°C. Additionally, the data suggest that the effect of 35°C incubation diminishes as exposure time to environmental stress increases. The higher MPN index suggests that growth in nonselective media at 35°C is necessary to maximize FC recovery in selective media at the higher temperature as noted previously by Geldreich et al. (16).

Using KF agar, the m-FS technique has been shown to give excellent recovery of FS from river water and sewage (8), the recovery being higher than the confirmed MPN (24). Since both the KF agar and ethyl violet azide broth permit enterococcus growth, the difference in recovery was attributed to growth of additional FS strains from nonhuman sources on the KF agar (24). In human feces, the FS densities obtained by the two methods are essentially the same (23). The m-FS counts on KF agar during this study were in close agreement with the confirmed MPN (Table 7), suggesting that nearly all of the FS were of human origin. This close agreement was not affected by the day samples were taken or by flow time from the pollution source. Although stresses in the aquatic environment have been shown to cause nonlethal cell injury which inhibits FS growth on selective media (2), the possibility of reduced recovery was not examined during this study because all FS media were selective.

TABLE 7. *Comparison of MPN and MF counts of FS per 100 ml from each sample station*

Sample station	Day sample taken	Confirmed MPN		MF	
		Index	95% Confidence limits	Density*	Verified
T-700	5	220	57–700	78	75
	12	240	68–750	160	160
	19	170	43–490	91	91
T-600	5	33	11–93	65	61
	12	23	7–70	80	67
	19	170	43–490	68	61
T-500	5	49	17–130	11	11
	12	170	43–490	40	40
	19	46	16–120	37	35
T-400	5	79	25–190	29	25
	12	49	17–130	27	25
	19	79	25–190	27	24
T-300	5	33	11–93	17	15
	12	33	11–93	26	25
	19	70	23–170	24	24
T-200	5	13	3–31	13	11
	12	33	11–93	22	21
	19	17	5–46	22	22
T-100	5	12	3–28	14	14
	12	33	11–93	20	19
	19	17	5–46	23	23

* Mean of three replicate portions.

The variability in TC, FC, and FS verification rates among the samples (Table 4) supports the recommendation that the percentage of verification from one sample should not be applied to any other sample (41). However, the data in Tables 5, 6, and 7 are indicative of what occurred throughout the sampling period and suggest that the MF technique provided an adequate measure of the TC and FS densities (Table 1). On the other hand, m-FC counts at all stations were suppressed, probably due to heat shock. In spite of the inherent problems, the MF technique is the most definitive method currently available for examining survival of these indicator bacteria.

Chemical parameters. The chemical parameters for all sample stations are shown in Table 8. The concentrations of several constituents at the C-100 station differed from the T-800 station, but the only measureable impact of the Chena River on the Tanana River was an increase in ammonia nitrogen. From T-700 downstream through T-100, calcium and sodium each showed a small but continuous concentration increase. The observed concentration changes between stations were minimal, and daily fluctuations at each station did not correlate with the variations in bacterial densities. This concurs with the observation by Geldreich et al. (15) that coliform survival at 10 and 20°C was not affected by wide variations of chemical contaminant concentration in stormwater. When McFeters and Stuart (30) examined *E. coli* suspensions in two streams, they found better survival at 4 to 6°C in the stream with the higher concentration of chemical constituents. Chemical parameter values in the Tanana River were even higher so it is possible that the chemical constituent concentrations may have had a direct effect on survival. Other studies (22, 32) suggest an indirect effect of chemical constituents because they found that higher concentrations increased the activity of indigenous predatory microorganisms. However, decreasing water temperature tends to limit predator activity (22), and there may be little or no activity at 0°C.

TABLE 8. *Comparison of chemical parameters in the water from each sample station*

Sample station	No. of samples	pH range	Conductivity range (μmhos)	Concn range (mg/liter)								
				Cl^-	Ca^{++}	Mg^{++}	Na^+	K^+	TC[a]	TOC[b]	NH_3-N	NO_3-N
T-900	5	6.7–7.4	220–291	1.7–2.0	42[c]	14–15	3.8–4.0	2.0–2.1	27–30	15–25	0.01	0.08–0.19
T-800	5	7.3–7.7	220–275	1.7–3.4	42	13–15	3.8–4.0	1.9–2.1	27–31	15–26	0.02–0.05	0.10–0.18
C-100	5	6.7–7.1	195–240	5.6–7.7	33–34	14–15	5.2–6.0	1.8–2.1	28–32	18–28	0.80–0.97	0.11–0.15
T-700	8	6.6–7.4	225–250	1.9–3.2	42	14–15	4.0	2.0–2.2	28–30	5–25	0.04–0.16	0.08–0.28
T-600	8	6.9–7.3	210–261	1.8–3.7	42–46	15–16	4.2–4.4	1.9–2.3	29–34	16–27	0.07–0.16	0.09–0.17
T-500	5	6.8–7.4	243–265	2.5–4.8	46	15	4.4–4.6	2.1–2.2	30–32	7–28	0.04–0.12	0.15–0.19
T-400	8	6.7–7.2	240–280	2.6–4.3	44–46	15–16	4.4–4.6	2.2–2.3	31–34	17–31	0.06–0.11	0.14–0.22
T-300	5	7.0–7.2	246–265	3.0–4.6	46	16	4.6	2.1–2.2	31–34	7–30	0.08–0.12	0.12–0.20
T-200	5	7.0–7.3	249–295	2.8–6.9	46–50	16–17	4.6–4.8	2.1–2.2	33–36	17–30	0.07–0.12	0.15–0.22
T-100	8	6.8–7.3	245–280	3.0–4.2	46–48	15–17	4.6–5.0	2.1–2.3	32–36	17–30	0.07–0.11	0.13–0.26

[a] Total carbon.
[b] Total organic carbon.
[c] Same concentration in all samples.

TC, FC, and FS survival. The discharges at stations T-800 and C-100 (Table 9), which monitor all effluent sources, indicated that the Chena River contributed a very small part of the water in the Tanana River. Survival was examined in the 317-km reach from T-700 downstream through T-100, and the dye study between stations showed that the mean flow time was 7.1 days, with a range of 6.0 to 9.1 days. Using the mean TC, FC, and FS densities (Table 1) and the discharge dilution factors (Table 9), with the densities at T-700 representing 100% viability, survival at stations T-600 through T-100 were calculated as shown by the following example:

[Mean density at T-600 × discharge dilution factor at T-600 ÷ mean density at T-700] × 100 = percentage of survival at T-600

The percentage of survival obtained at each station was plotted versus mean flow time to obtain the survival curves (Fig. 2). These data indicate that the most rapid reduction in numbers occurred during the first 1.9 days of travel time, with 38.0% TC, 42.5% FC, and 65.9% FS remaining at T-600. Die-away continued at a slower rate, with 8.4% TC, 15.7% FC, and 32.8% FS remaining viable at the T-100 station after 7.1 days. The rapid density decrease followed by a slower decline during subsequent days has been reported in warmer waters (5, 7, 15, 25) and 0°C in the Tanana River. The relative survival is TC < FC < FS at all stations, which is substantiated by the decreasing TC-FC and FC-FS ratios (Table 3). The much higher survival rate for the FS than for either the TC or FC supports previous findings (7, 18, 29). Variable results for the relative TC and FC survival have been reported (11, 15, 18, 29) and may be caused by experimental or environmental differences.

Although an initial increase in coliform numbers before onset of the rapid decrease has been observed at temperatures above the minimum for coliform growth (5, 25), the 0°C temperatur would have prevented aftergrowth during this study. It has been suggested (2, 25) that the biphasic survival curves result from two subpopulations, one which does not survive well in the aquatic environment and one that is more persistent. A third population appears to exist and may be a significant portion of the FC population, but does not grow on selective media (2). In a river, it is difficult to ascertain the actual size and importance of the third subpopulation because of the presence of a mixed bacterial population capable of growth on nonselective media.

Fecal indicator bacteria survival during this study is not directly comparable with the earlier Tanana River study (18) because ice surface conditions which prohibited aircraft operation made it necessary to relocate stations T-500, T-400, T-300, and T-100. Also, the dye study indicated that the mean flow time from T-700 and T-600 was 1.9 days instead of the 1.2 days previously estimated. However, the overall survival curves were similar. On this basis, little difference was found in TC survival although the percentages in this study indicate a slightly greater survival than the maximum values reported previously. In contrast, the evidence suggests that the highest survival rate reported in the prior study significantly underestimated FC viability. The percentages reported here are higher than those previously reported by 1.7 times at T-600, increasing continuously to 3.7

TABLE 9. *Hydrological data from sample stations on the Tanana River*

Sample station	Discharge			Distance between stations (km)	Days flow time between stations		
	Vol (m^3/s)	Measurement error[a]	Dilution factor		Minimum	Mean[b]	Maximum
T-900	[c]						
T-800	161.4	±5–8%					
C-100	6.5	±5%					
T-700	172.2	±5%	1.80	77	1.5	1.9	2.5
T-600	175.5	±5–8%	1.02	46	0.8	1.0	1.5
T-500	234.5	±5%	1.36	56	1.1	1.3	1.6
T-400	247.8	±5%	1.44	59	1.0	1.1	1.4
T-300	294.5	±5%	1.71	42	0.9	1.0	1.1
T-200	277.5[d]	±5–8%	1.71	46	0.7	0.8	1.0
T-100	294.5	±5%	1.71				

[a] Judgement of hydrologist at time discharge measurement was made.
[b] Time required for 50% of the dye to pass.
[c] Should be essentially the same as measured at the T-900 station.
[d] Probably low because of meter icing and is considered to be the same as measured at the T-100 and T-300 stations.

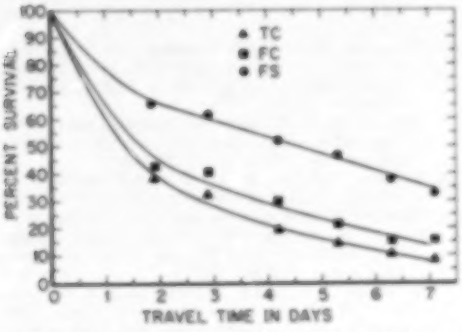

FIG. 2. *Percentage of survival of fecal indicator bacteria in the T-700 to T-100 reach of the Tanana River.*

times at T-100. Survival of FS in this study was very similar to the earlier study, with the percentages generally lower than the maxima reported previously.

The extended fecal indicator bacteria survival reported here and in other ice-covered river studies (11, 18) suggests that maximum survival under natural conditions occurs in 0°C water under ice cover. Survival of enteric pathogens is also expected to be enhanced under these conditions, and *Salmonella* serotypes have been recovered from the Tanana River sample stations during the winter (43). To elucidate the survival characteristics of enteric pathogens, samples obtained during this study are being examined qualitatively and quantitatively for *Salmonella* serotypes and enteric viruses. In addition, pure cultures of TC, FC, and FS isolated from each sample station are being identified to determine if there are differences in survival characteristics of specific group members.

Recently, Dutka (9) pointed out that there is a continuing need for indicators, although a quantitative relationship does not exist between coliforms and enteric pathogens. Among the reasons for the lack of a quantitative correlation in receiving streams are coliform aftergrowth and intermittent excretion of pathogens. Since aftergrowth is not a problem at 0°C in an ice-covered receiving stream, relative survival characteristics should provide a more definitive correlation between indicator bacteria and the probable presence of enteric pathogens at low water temperatures.

ACKNOWLEDGMENTS

We are indebted to Ruth McFadden for providing laboratory support, Robert Jackson for conducting the dye study, Steve Swingle and Paul Doyle (U.S. Dept. of Interior, U.S. Geological Survey, Water Resources Division) for providing the discharge measurements, Robert Bell for field logistical support, James Pickering and Roger Oppedahl (U.S. Dept. of Interior, Office of Aircraft Services) for many hours of flying and help in the field, and Carolyn McClintock for secretarial support. We also wish to express our appreciation to the other personnel at the Arctic Environmental Research Station for their tolerance and support.

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